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PATENT

Attorney Docket No.: 27373/39055B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Wang *et al.*
Application No. 09/748,710
Filed: December 22, 2000
For: METHOD FOR
GENERATION OF LONGER
cDNA FRAGMENTS FROM
SAGE TAGS FOR GENE
IDENTIFICATION
Group Art Unit: 1637
Examiner: Joyce Tung

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Date: February 19, 2004

Mail Stop AF,
W.K.M.
2/18/04

William K. Muehl

**DECLARATION OF SAN MING WANG, JIAN-JUN CHEN, AND JANET ROWLEY
UNDER 37 C.F.R. § 1.131**

We, San Ming Wang, Jian-jun Chen, and Janet Rowley, state that,

1. We are the inventors of the subject matter claimed in the above-identified application, which claims priority to U.S. Provisional Application Nos.: 60/174,391 and 60/173,617, filed January 3, 2000, and December 29, 1999, respectively. We are making this declaration to provide evidence that the subject matter claimed in the above-identified application was completed in the United States at a date at least prior to July 19, 1999, which is the date of acceptance of van den Berg, et al., Nucleic Acids Research, 1999, Vol. 27(17), pages i-iii, cited by the Examiner in an Office Action mailed March 26, 2003.

2. To establish the date of completion of the invention, copies of pages from San Ming Wang's laboratory notebook are attached as Exhibit A. All dates appearing on these laboratory notebook pages have been redacted.

3. The laboratory notebook pages establish the following facts:

identifying (a) Information from SAGE tags alone resulted in problems in some genes;

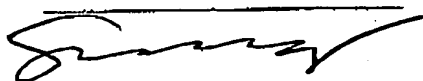
extend (b) The solution to the problems associated with SAGE tags was to the SAGE tag sequence to generate a longer, more specific 3' cDNA probe;

in (c) The longer probe could be generated using a SAGE tag as a primer an extension and/or amplification reaction(s);

is (d) One amplification reaction useful in solving the SAGE tag problem PCR, using a SAGE tag primer and some form of universal primer, such as an oligo (dT) primer, optionally containing an anchor, with a cDNA as a substrate;

4. These laboratory notebook pages confirm that the subject matter of the pending claims in the application was completed at least prior to July 19, 1999.

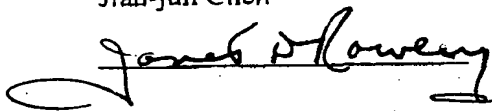
5. Each of us hereby declares as follows: All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true and, further, these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



San Ming Wang, Ph.D.



Jian-jun Chen



Janet Rowley

2-17-2004
Date

2-18-2004

Date

2-18-2004

Date

select candidate genes from different classes based on the tag sequences from Lin Zhang, John Hopkins.

• Confirm the matched genes for tag seq. by Blastn parameter set: expect: 1000, cutoff: 60.

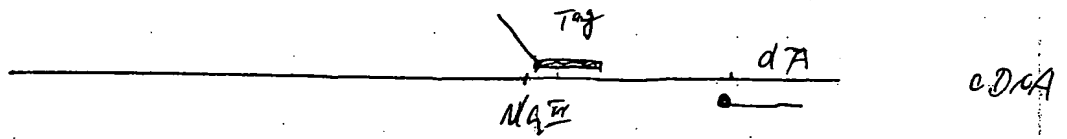
sequences aligned show many aligned genes in many case. to define the most likely ones, it needs to stress:

a. 5' add CATG

e.g. 5' CATG XXXXXXXXXXXX 3'
tag

b. the matched sequences should be located in the last N/4 site of the sequence

c. confirm the right gene by ^{generally} using large template based on the tag sequence. This is not available in SAGE technique. I designed a system to stress this issue. If solved, it will largely improve the reality of ~~seq~~ gene identification in SAGE.



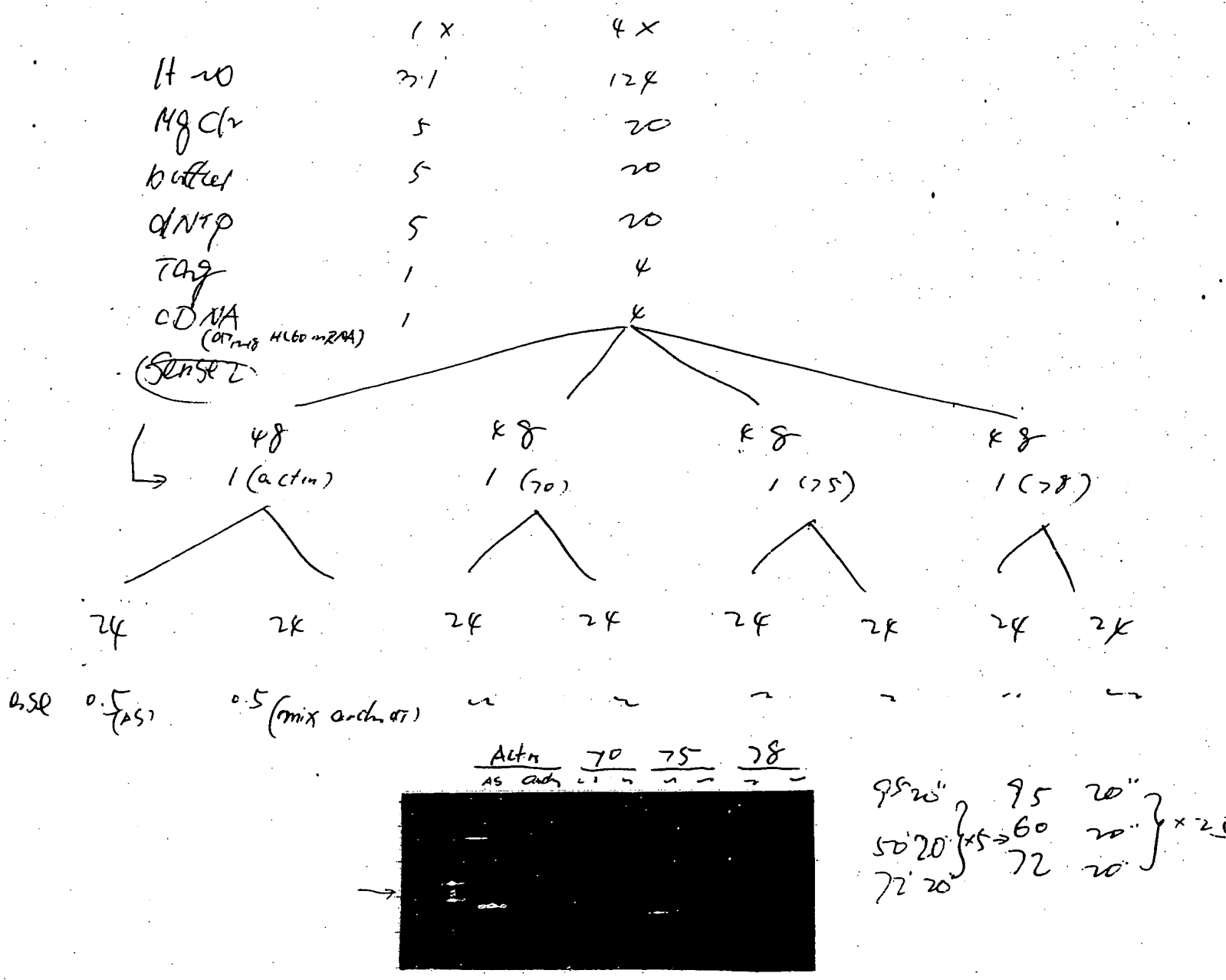
- based on Tag seq. design 5' seq sense primer, plus 6 bases tail to maintain the stringency

anchored dT
It is known well
Tag pol
closely distinguish
2 anchored primer
reactions

- use anchored primer as 3' antisense primer to select 3' subpopulation for PCR. IMPORTANT! NEW IDEA
- PCR amplify cDNA
- the cDNA should be pre-digested by N/A. The possible outcome would be
- specific temp amplified because of the 5' sense primer / 3' anchored primer gives exponential amplification, others will only be ~~double~~ increased. 1, 2, 3, 4... potential problem ~
- amplification efficiency lower, due to 3' extension consume large portion of substrates
- specificity?

IED model system to confirm the possibility

genes β -Actin S2/AS S2/Act, S2/GAT
 HSC 70 mixture of Act, A/G/C
 HSP 75
 HSP 78



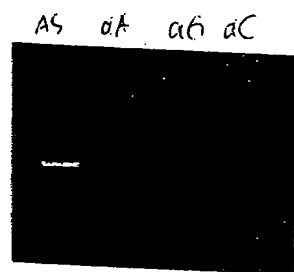
conclusion. the mixture of anchored primers doesn't provide

The possible reason for failure to ~~do~~ amplify may be that the mixture of anchored $\alpha 7$ disturbed the efficient amplification. Try to use separated anchored $\alpha 7$ as 3' primer. use ACT1 as model

	2x
H ₂ O	6.2
MgCl ₂	10
buffer	10
dNTP	10
Taq	2
cDNA	2
Sense (actin)	2

15' 25' 25' 25'

0.5 (AS) 0.5 (dATn) 0.5 (dG Tn) 0.5 (dCTn)



β -actin mRNA end (HSAC07)

HAAGTG CACAC CTTA TTTTTT
 A TTTTTTTT (dA dTn)

Conclusion (1) dA anchored as 3' OK

Improve:

- increase 3' anchor primer amount
- decrease first 5 cycles in PCR to allow 3' primer bind to temp, then shift to 60°C for annealing
- increase cycle number to 40 of Actin

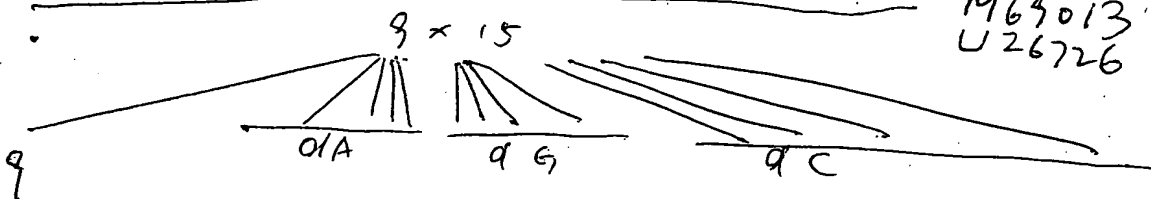
	8x
Myc/2	40
buffer	40
QNT/2	40
Taq	8
CDNA	8
Sense	8

QATn
QATn
QCTn

ef1- α
ribosomal
C37 α
S24

X037K7
M63013
U26726

AA5142
A219316
S28
AA67692
AA61797
AJ223473
D25786



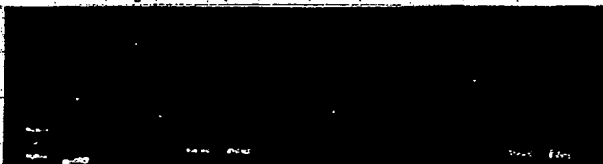
Sense 1 1 5 10 15 15 10 15 1 5 10 15
50ng 250ng 500ng 750ng

no 14 14 10 5 - 14 10 5 - 14 10 5 -

25ml

94°C 20' }
42°C 20' } x5 → 60°C 20' } x30
72°C 20' }

S/As AIn GIn CIn
50ng 250ng 500ng 750ng

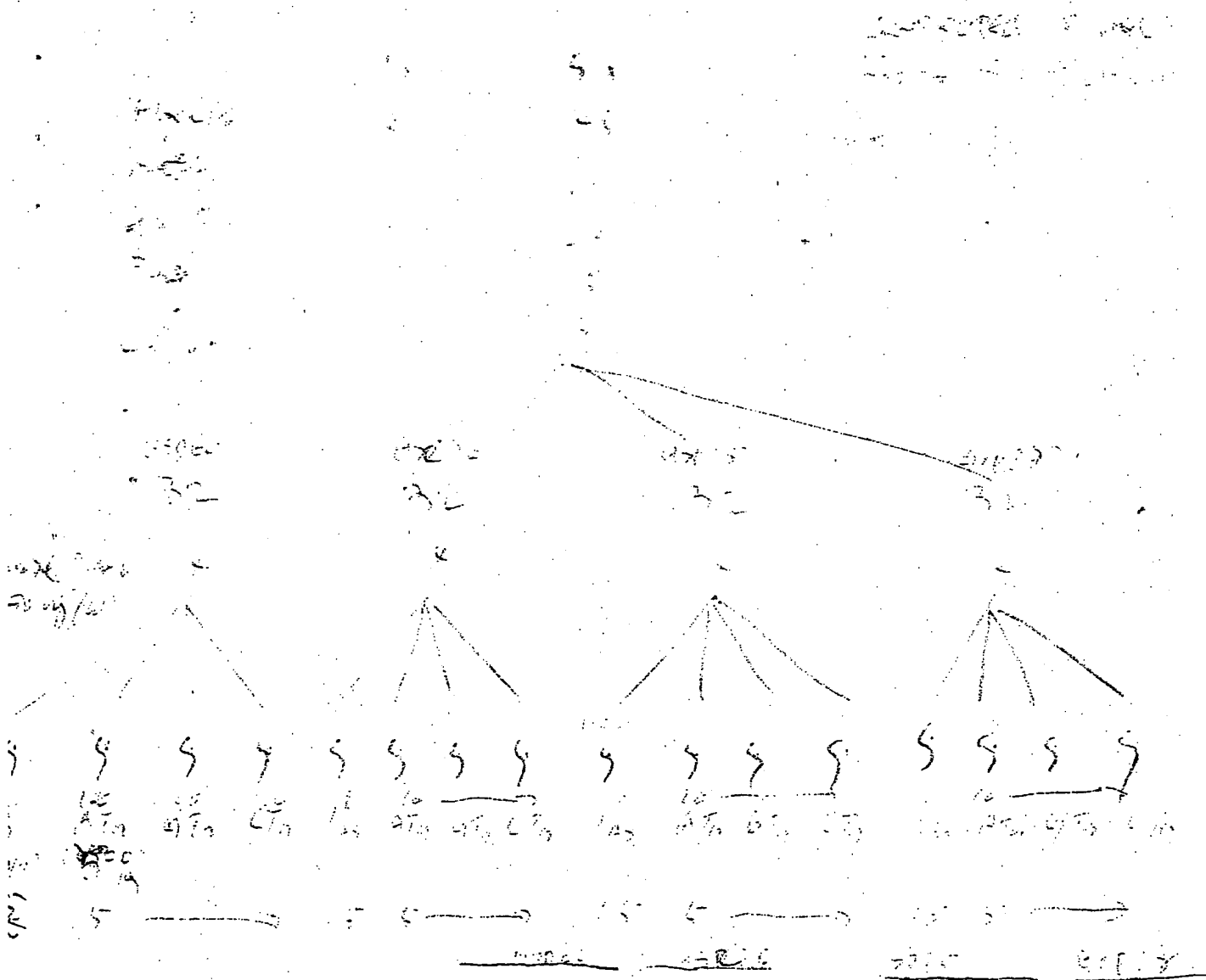


Conclusion:

- ① increase anchor primer amount did increase the amplification
- ② CTn still generated diffuse in principle, this strategy should work! Can be used

ACTED

to test if the style some principle of the and
for other template like at lower level
complexity of the

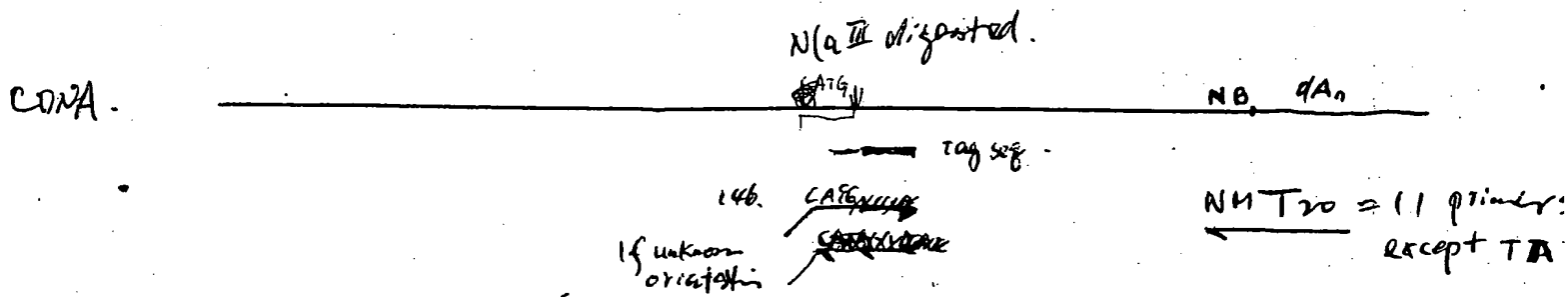


* Also, the cDNA should be generated with anchor set not digested which creates background in PCR.

It seems that one base anchored dT primers may not generate enough specificity in particular temp, due to the complicated cDNA pool composition, or different members in the same family like Hsp70s.

To increase the specificity, it may be useful to use 2 base anchored dT, set as 3' antisense primers.

The complete set of sense / antisense will be



to identify each temp tag gene, the total reaction will

be 11 PCR reaction with Tag seq as sense (+ 6 base^{5'} to

if negative, reversed Tag sequence will be used as the sense primer in case the original tag was generated in 3'-5' direction.

in this way, the specific 3' fragment will be generated, and match to database.

try repeat exp. with anchor primer generated cDNA → {one base anchor 5' 5'}